



Title	Signalling by protein phosphatases and drug development: a systems-centred view
Authors(s)	Nguyen, Lan K., Matallanas, David, Croucher, David R., et al.
Publication date	2012-03-14
Publication information	Nguyen, Lan K., David Matallanas, David R. Croucher, and et al. "Signalling by Protein Phosphatases and Drug Development: A Systems-Centred View." Wiley Blackwell (Blackwell Publishing), March 14, 2012. https://doi.org/10.1111/j.1742-4658.2012.08522.x .
Publisher	Wiley Blackwell (Blackwell Publishing)
Item record/more information	http://hdl.handle.net/10197/5577
Publisher's statement	This is the author's version of the following article: Lan K. Nguyen, David Matallanas, David R. Croucher, Alexander von Kriegsheim, & Boris N. Kholodenko (2012) "Signalling by protein phosphatases and drug development: a systems-centred view" FEBS Journal, 280 : 751-765 which has been published in final form at http://dx.doi.org/10.1111/j.1742-4658.2012.08522.x .
Publisher's version (DOI)	10.1111/j.1742-4658.2012.08522.x

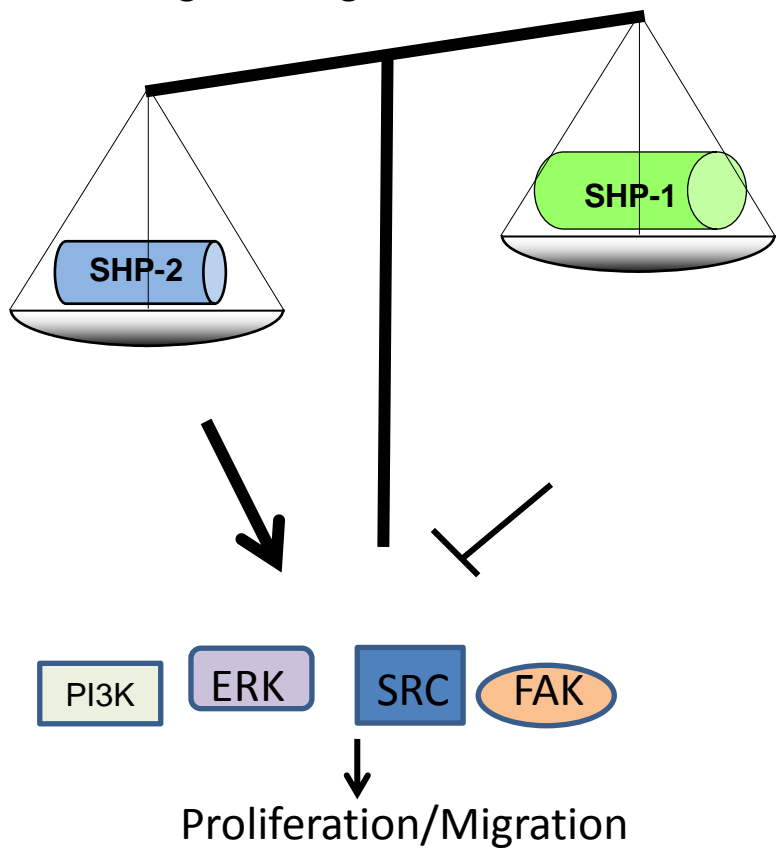
Downloaded 2026-05-01 23:46:11

The UCD community has made this article openly available. Please share how this access benefits you. Your story matters! (@ucd_oa)

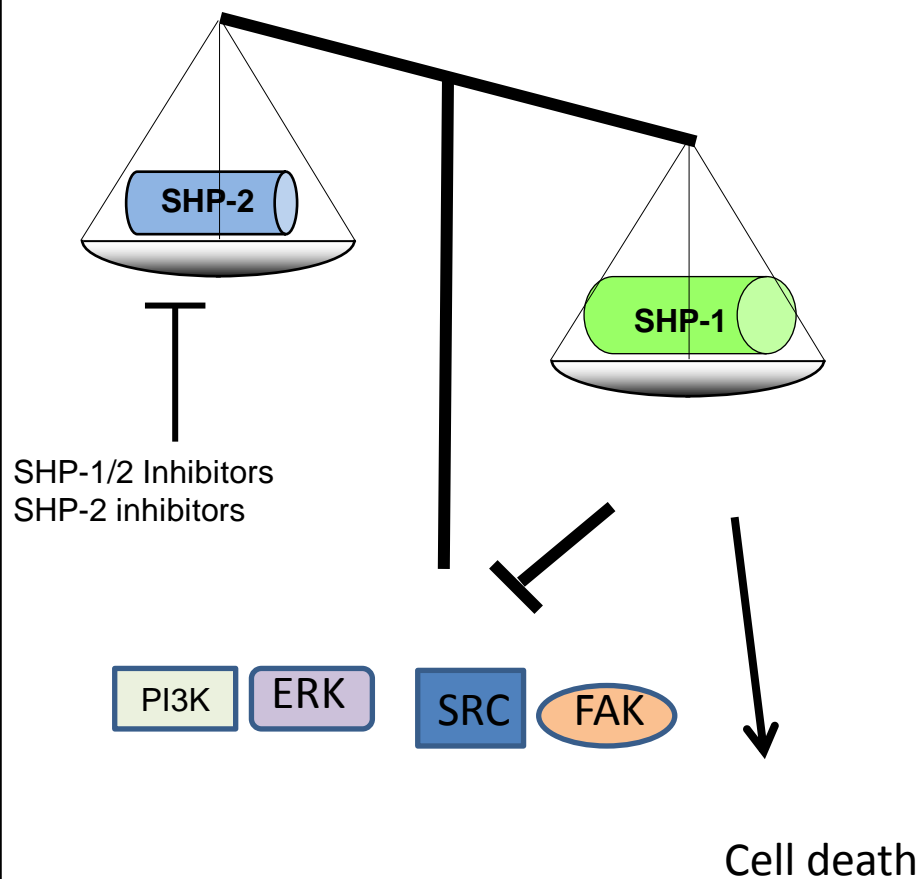


© Some rights reserved. For more information

A Growth factor and cytokine stimuli or oncogenic signals

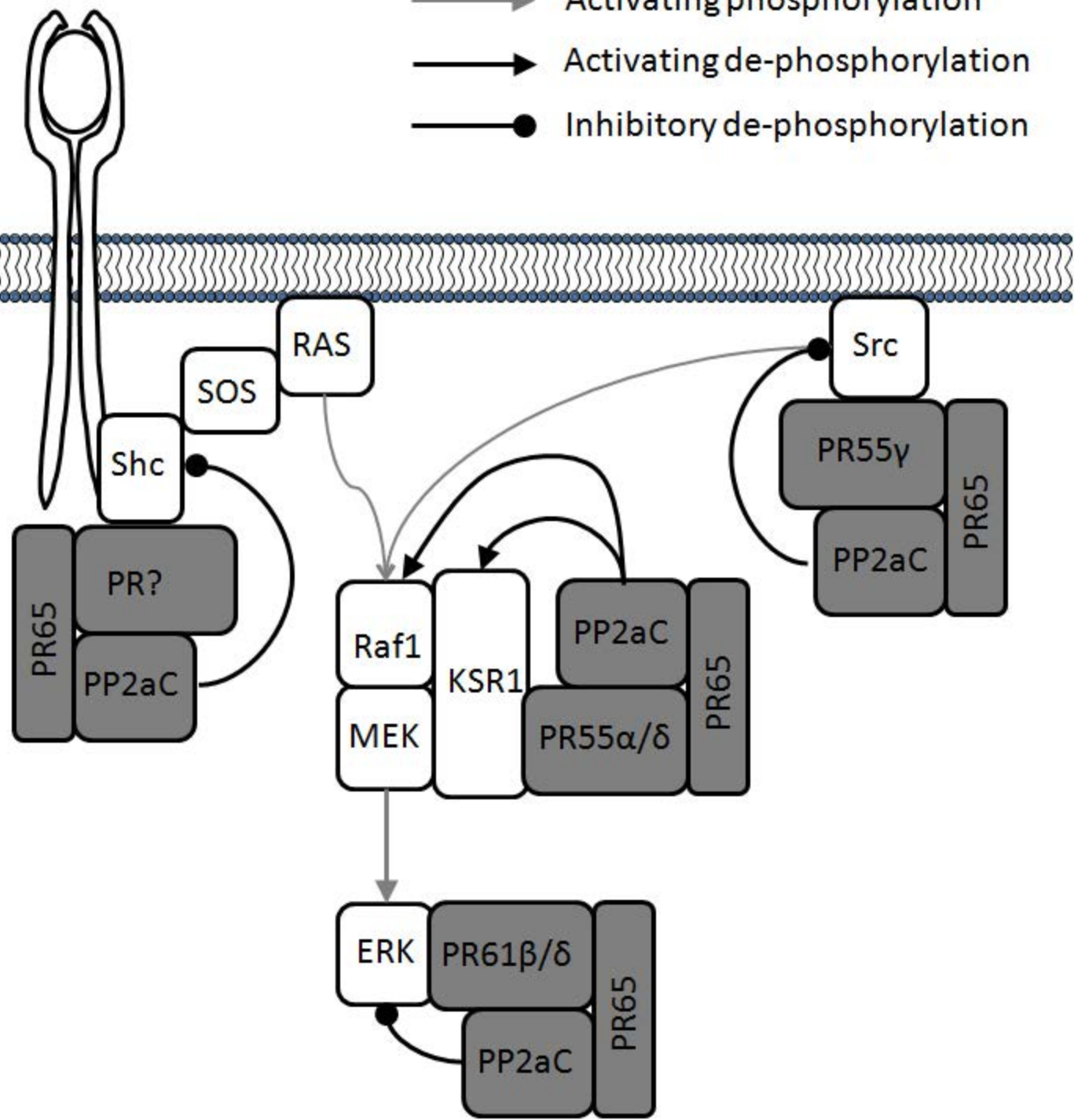


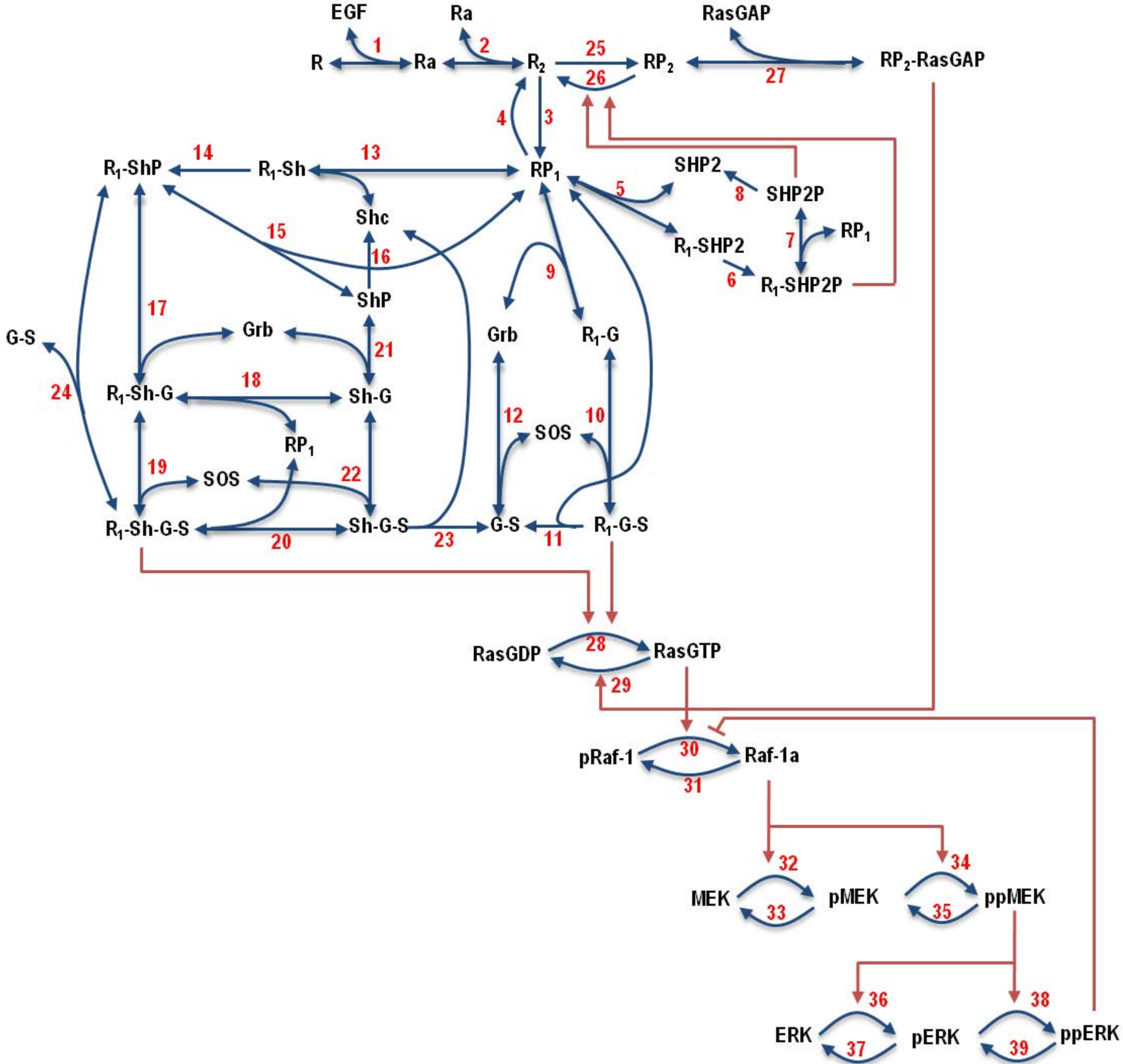
B Growth factor and cytokine stimuli or oncogenic signals

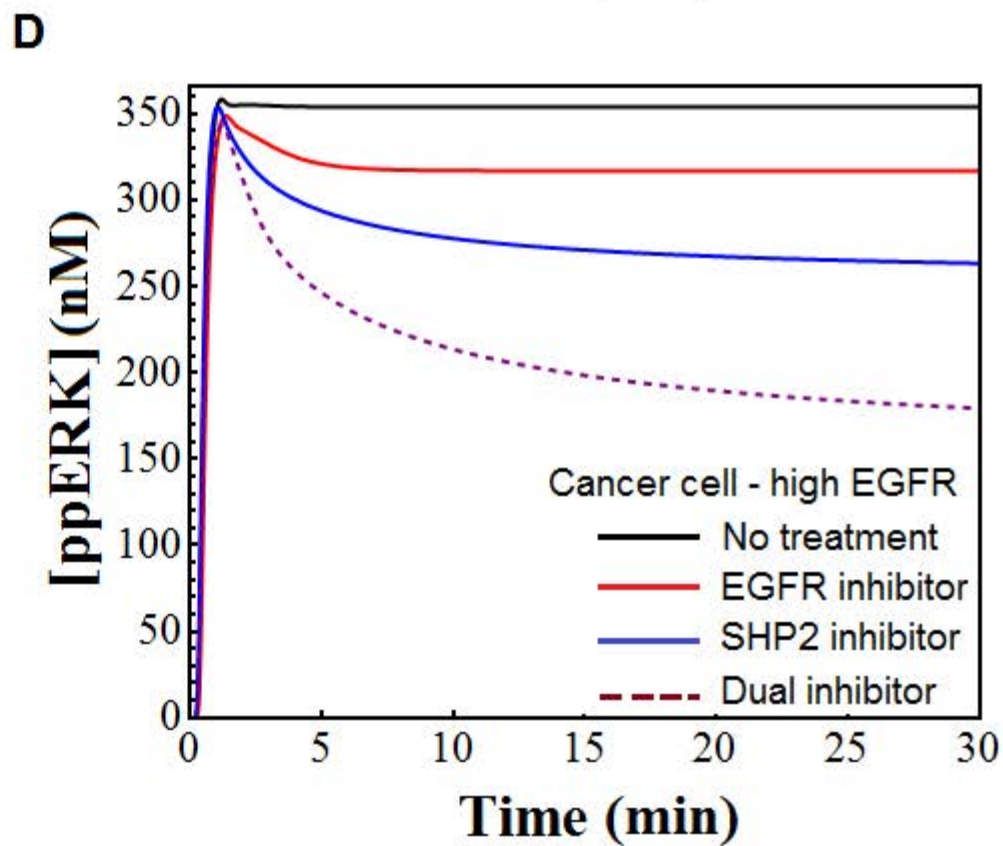
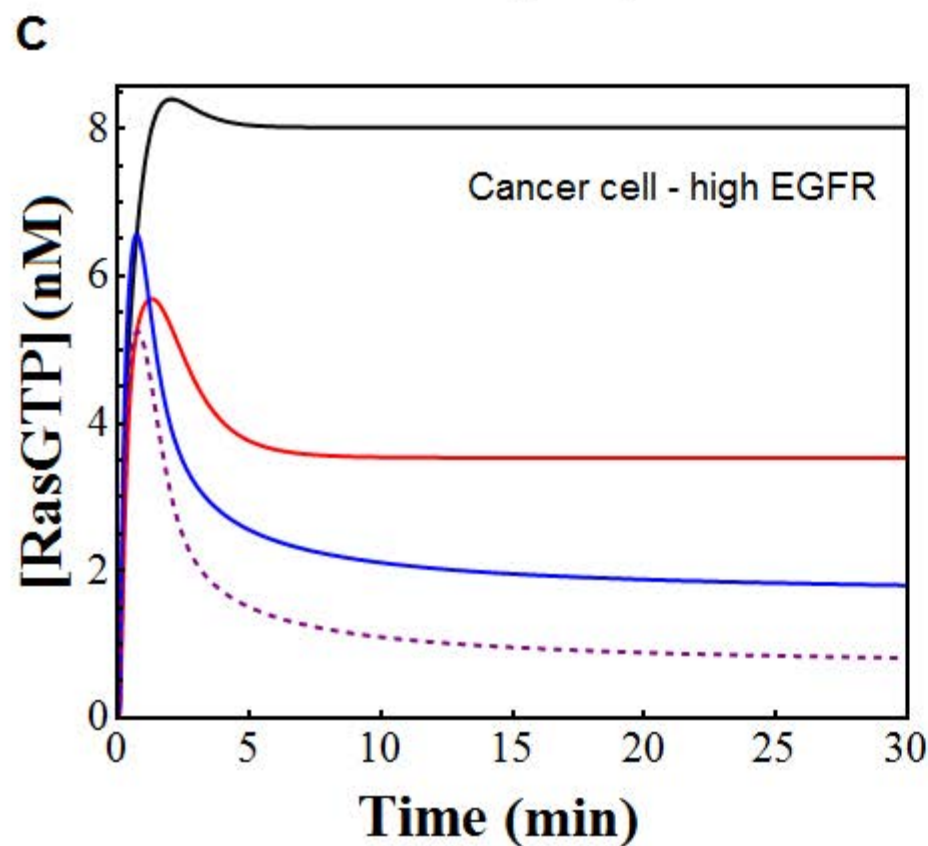
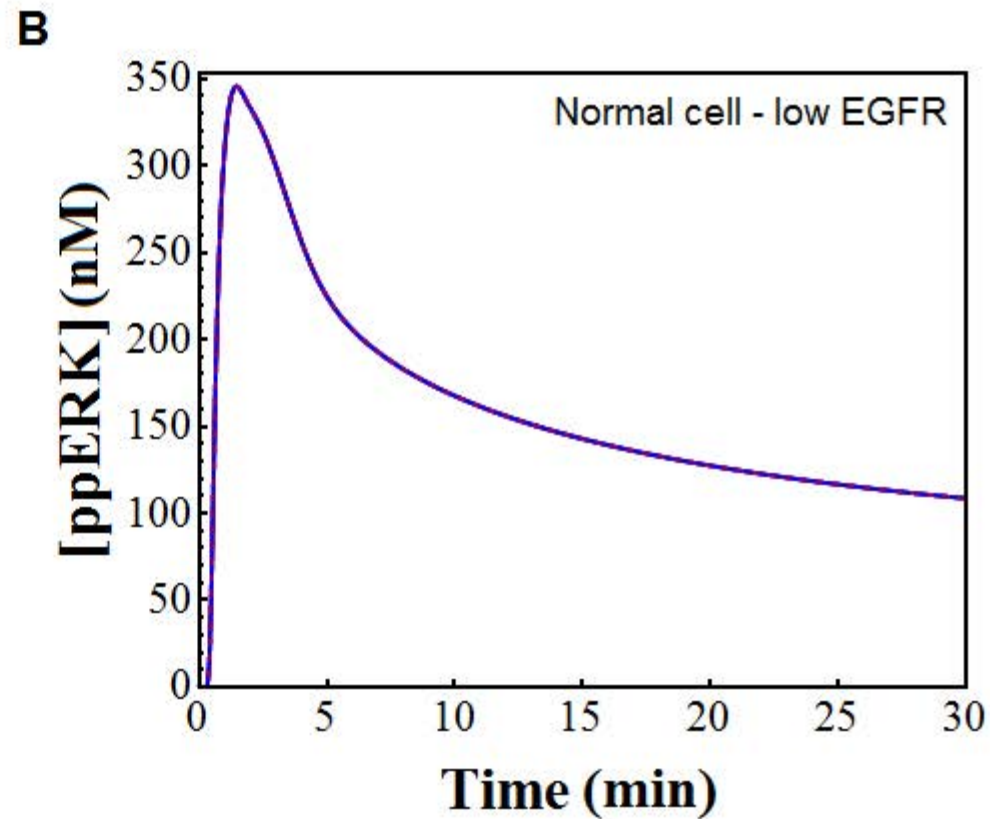
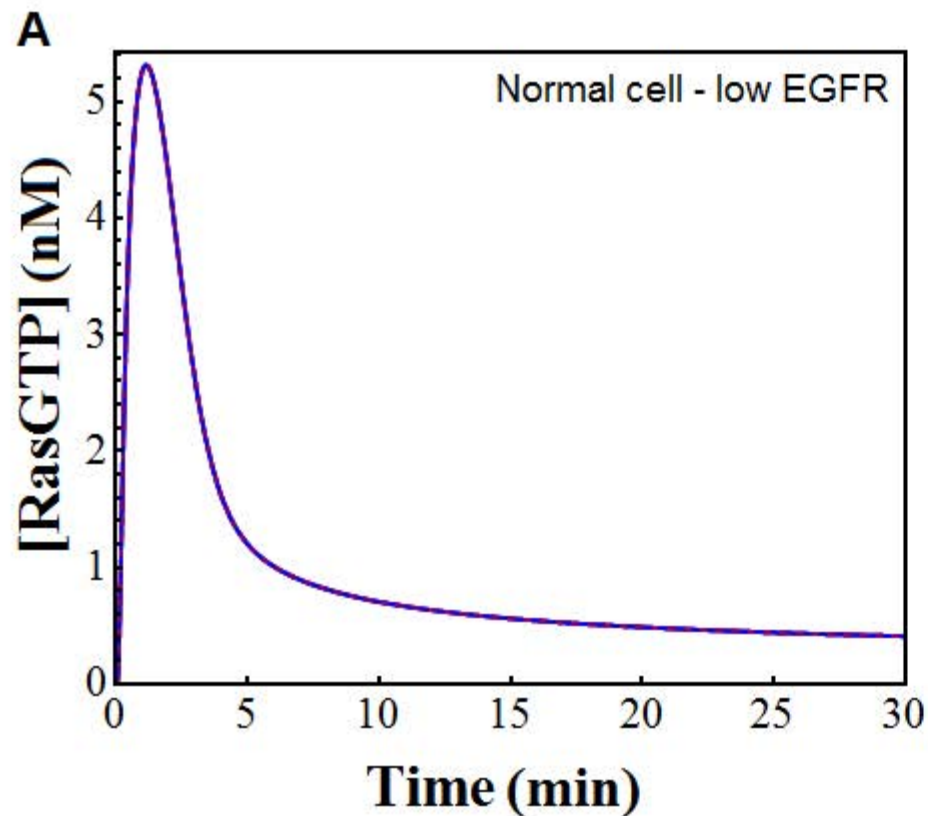


Activated
RTK

- Activating phosphorylation
- Activating de-phosphorylation
- Inhibitory de-phosphorylation







Signalling by protein phosphatases and drug development: a systems-centred view.

Lan K. Nguyen^{1*}, David Matallanas^{1*}, David R. Croucher^{1*}, Alexander von Kriegsheim^{1*} and Boris N. Kholodenko^{1#}

Systems Biology Ireland, University College Dublin, Belfield, Dublin 4, Ireland

Abstract

Protein-modification cycles catalysed by opposing enzymes, such as kinases and phosphatases form the backbone of signalling networks. Whereas historically, kinases have been at the research forefront, a systems-centred approach reveals predominant roles of phosphatases in controlling the network response times and the spatiotemporal profiles of signalling activities. Emerging evidence suggests that phosphatase kinetics are critical for the network function and cell-fate decisions. Protein phosphatases can operate as both immediate and delayed regulators of signal transduction, capable of attenuating or amplifying signalling. This versatility of phosphatase action emphasises the need for systems biology approaches to comprehend cellular signalling networks and predict the cellular outcomes of combinatorial drug interventions.

*These authors contribute equally.

#Correspondence to Boris N. Kholodenko: boris.kholodenko@ucd.ie

Keywords: phosphatases, spatiotemporal dynamics, signalling cascades, systems biology, drug discovery

Short running title: Phosphatases and drug development: a systems-centred view

Introduction

Multiple external cues including growth factors, cytokines and mechanical forces activate plasma membrane receptors such as receptor tyrosine kinases (RTKs), G-protein coupled receptors and other receptor families. This creates spatiotemporal phosphorylation patterns which propagate through a wired network of signalling proteins and cascades. Frequently combined with intrinsic intracellular signalling, this tangled network not only transmits, but also processes and decodes the external information and gives rise to cellular response. For instance, signals from different receptors are integrated through common targets and pathway crosstalk, such as in cell cycle control [1, 2] or mTOR-mediated signalling [3]. Phosphatases play a vital role in cellular signalling by controlling both the network dynamics and spatial localisation of phosphoproteins [4, 5]. Although kinases have previously been in the limelight of scientific interest, protein tyrosine phosphatases (PTPs) and Ser/Thr phosphatases (PSPs) are becoming increasingly explored both as a research topic and targets for drug development.

Phosphatases act as both immediate and delayed negative regulators of protein phosphorylation, and this often results in attenuation or termination of signal transfer. Constitutive phosphatase activities shape the initial phosphorylation profiles of receptors, phosphorylated adaptors, Ser/Thr kinases and other signalling proteins, following transient activation by growth factors or other signals. These immediate signalling responses develop very rapidly on the scale of second and minutes, and as we will see below phosphatases can play a dominant role in setting the spatiotemporal behaviour of protein phosphorylation in the cell. Induced phosphatase activities often create negative feedback loops, which adapt cells to a more permanent external stimulation, persisting on the timescale of hours. Interestingly, inhibition of PTPs by reactive oxygen species induced by activated RTKs, such as epidermal growth factor receptor (EGFR) can create also positive feedback loops that facilitate the lateral propagation of EGFR phosphorylation at the plasma membrane [6]. This multifaceted role of phosphatases in the signalling control is illustrated by many examples, for instance, by the dual role of the SH2-domain containing protein tyrosine phosphatase-2 (SHP2) in activation of the extracellular signal regulated kinase (ERK) which we discuss in detail below [7-9].

Compared to kinases, the progress towards understanding of the regulation of phosphatases was lagging behind due to technical challenges. For example, it is only possible to assay the activity of a given protein phosphatase *in vitro* if one already has the relevant substrate phosphorylated by a relevant protein kinase. In fact, in the human genome the numbers of different PTPs and RTKs are very similar, implying that versatility and specificity of the functions of these kinases and phosphatases can also be similar [10]. Although catalytic subunits of PSPs have overlapping targets, the substrate specificity of PSPs is often achieved through their regulatory subunits [11, 12]. Different regulatory and scaffolding subunits recruit a catalytic subunit to specific sub-cellular locales where different targets reside. Individual ternary PSP complexes assembled in these locales have differential catalytic activities and endow a particular PTP with proper substrate specificities. In this review we focus on how substrate specificity is controlled for phosphatases of the PSP family.

Historically, kinases have been major drug targets for cancer and other diseases. However, versatility of phosphatase functions and their involvement in multiple feedback mechanism makes phosphatases attractive targets for future drug development. We will discuss how PSPs are advancing to the forefront of drug development. To demonstrate the potential of systems biology approaches in facilitating the selection of therapeutic targets, we develop a simplified mathematical model of the EGFR/SHP2 signalling pathway and explore

in silico phosphatase-based therapies vs receptor inhibition. Both theoretical and experimental studies focusing on understanding roles of phosphatases in controlling the spatiotemporal dynamics of signalling networks will be discussed. We will also show how phosphatase dynamics are regulated by the transcriptional machinery and how such transcriptional feedback loops control the entire signalling system in the context of mitogen-activated protein kinase cascades.

Phosphatases shape temporal dynamics of signalling cascades

Signal transduction via cascades of phosphorylation/dephosphorylation cycles is a hallmark of cell signalling. The highly conserved mitogen-activated protein kinase (MAPK) cascades, which have been extensively studied, control a range of important physiological processes, including proliferation, differentiation and apoptosis [13, 14]. MAPK cascades consist of three sequential levels, with phosphorylation and subsequent dephosphorylation catalysed by a kinase from a preceding level and a phosphatase at a given level, respectively.

Activity of signalling cascades such as the MAPK network can be characterised by a number of key features, notably amplitude and duration of the signal output, both of which bear relevant physiological impact. Signal amplitude of MAPK activation exceeding a certain threshold was found as a requirement for the proliferation of fibroblasts [15]. While on the other hand, the duration of MAPK activity in PC12 cells dictates whether the cells would proliferate or differentiate [16]. Moreover, rapid and transient MAPK activation in rat hepatocytes promotes the G1-S cell-cycle progression while prolonged MAPK activation inhibits this process [17]. By influencing different repertoires of target genes, the amplitude and duration of MAPK activation are critical in determining cell responses [16-19], and thus their quantitative description can be used to gain insights into differential roles of the participating phosphatases and kinases in shaping the cascade signalling outputs.

Theoretical analysis of signalling cascades without feedback loops has shown that the action of phosphatases outweigh that of kinases, exerting a dominant effect on the regulation of signal duration [5]. On the other hand, kinases influence signal amplitude rather than duration, although phosphatases can also contribute to the regulation of signal amplitude. This is particularly apparent in weakly activated pathways where only a small proportion of the total kinase pool is phosphorylated. Under these conditions, signal duration is entirely determined by phosphatases, becoming prolonged at slow dephosphorylation rates. Interestingly, the position of a phosphatase within the cascade does not affect the extent to which it affects signal duration [5]. Mathematical studies on specific systems such as the ERK pathway have provided further support to these predictions [20, 21]. In one such study utilising NRK fibroblasts [20], the cells were arrested in G₀ phase and ppERK concentrations were measured following stimulation with EGF in the presence of increasing doses of a MEK inhibitor [20]. Under these conditions, increasing MEK inhibition resulted in a decreased peak of a transient ERK activation, while having little effect on its duration. However, applying a protein tyrosine phosphatase (PTP) inhibitor led to a broader ppERK peak, signifying a prolonged duration which is consistent with model predictions [20]. These studies suggest that in signalling pathways such as the MAPK cascade, where signal duration strongly determines cell fates, targeting phosphatases rather than kinases is a more viable strategy to control cell responses.

Dual-specificity phosphatases (DUSP) as rapid feedback inhibitors As mentioned above, MAPK pathway signalling has been implicated in the governing cell fate decisions. Diverse cellular events, such as proliferation, differentiation, migration and apoptosis all require the proper functioning of MAPK cascades. A puzzling aspect has been of how one core module, such as the Ras/Raf/MEK/ERK pathway can elicit cell responses as

diametrically opposite as proliferation and differentiation. It has emerged over time that the answer to this question is due to the fact that these pathways, which were traditionally thought to be linear cascades, are embedded in complex signalling networks of feedback interactions [14, 22, 23]. When a signal is relayed from the extracellular membrane via the MAPK-pathway into the nucleus, a networked pathway allows for additional regulation by either integrating information from alternative co-activated and suppressed pathways or by facilitating the self-regulation of the pathway by incorporating feedbacks. Although other classes of protein phosphatase, such as protein serine/threonine phosphatase 2A (PP2A) also have direct or indirect regulatory effects on the MAPK cascade, in this part of the review we will focus on how DUSPs elicit feedback control in the context of the Ras/Raf/MEK/ERK pathways.

In response to extra and intracellular signalling cues, cells induce regulatory feedbacks by two essentially different mechanisms. Either the activities of signal transducers are altered, or protein concentrations of these transducers are changed. The activity changes are generally achieved post-translationally through altering modifications, such as phosphorylations that occur rapidly and at multiple levels of the pathway. For instance, ERK alone can phosphorylate and inactivate several upstream signal transducers, including EGFR, SOS, Raf-1 and MEK [14]. Such feedback controls mediated by post-translational modification occur almost immediately after the initial signal has been triggered. Furthermore, protein concentrations can be changed by either increasing protein degradation, such as depletion of the EGF-receptor [24], or by triggering a rapid transcriptional response, which can be induced on the time scale of minutes. Many genes, which are strongly induced in this first transcriptional wave, are direct regulators of upstream signalling, showing that biological systems exploit both post-translational and transcriptional feedbacks. Nevertheless, transcriptional feedbacks are inherently slower and more costly than post-translational ones, as it takes time and energy to induce substantial amounts of a nascent protein. Therefore, increasing the concentrations of feedback inhibitors has a delayed effect on the signalling cascade and this inhibition is frequently sustained for longer periods. The increase of feedback inhibitor concentration allows the cellular system, which is initially very sensitive to extracellular cues, to adapt to the new environment. This is done by adjusting the threshold required for signalling, by reducing the signalling sensitivity and by altering the dynamics of the response. The adaptation can involve reducing the receptor abundance [24], expressing specific antagonists, such as Sprouty [25] and MIG-6 [26] or inducing the expression of phosphatases, which dephosphorylate activating phosphorylation sites on signal transducers [27, 28]. One class of phosphatases, which is robustly induced upon activation of MAPK cascades, are dual-specificity phosphatases, reviewed in this journal edition by Steve Keyes. DUSPs are a subfamily of PTPs which bind MAPKs and dephosphorylate residues in their activation-loop leading to MAPK deactivation. Interestingly, DUSP activity is additionally regulated by the substrates, and binding to MAPK increases DUSP activity [29]. Additional regulation is achieved through post-translational modifications, as many DUSPs are themselves substrates of MAPK [30]. Therefore, inducible over-expression of DUSPs potentially decreases MAPK-activity and is considered to be part of the cellular feedback mechanism.

These feedback inhibitors are required to respond rapidly and with sufficiently high precision to changes in MAPK activity. Rapid turnover times are achieved through fast protein and mRNA degradation rates, which are hallmarks of these feedback regulators [31]. This allows for a rapid transcriptional regulation of the protein, which in turn permits the accurate and reliable tuning of the signalling response.

Induced expression of phosphatases reduces the dose-response sensitivity and the

signalling output, but can also fundamentally change the dynamics of the response. For instance, NIH-3T3 cells show a rapid and sustained phosphorylation of the downstream ERK1/2 kinases following stimulation with platelet-derived growth factor (PDGF) [32]. Interestingly, the pathway activation appears to be self-sustaining, as MAPK activity persists even when PDGF is washed out after the initial stimulus. Under these conditions, ERK1/2 activity is not linearly related to the PDGF input concentration. Incremental increasing PDGF concentrations does not lead to incremental increases in MAPK signalling, but rather results in a switch-like, all-or-nothing activation above a certain threshold, similar to an ultrasensitive activation. Although DUSP is induced by PDGF, this expression fails to dramatically affect ERK1/2 activation dynamics [32]. Although post-peak MAPK activity is reduced, the sustained MAPK activation dynamics still persists. Intriguingly, the behaviour of the system can change dramatically if cellular DUSP expression is substantially increased by pre-exposing NIH-3T3 cells to low PDGF concentrations. Re-stimulating these preconditioned NIH-3T3 with increasing PDGF concentrations dramatically changes the dose response sensitivity of the MAPK activity. The previously ultrasensitive system now displays a linear relationship between the PDGF input and the MAPK phosphorylation output. The transformation of a switch-like ultrasensitive response to a graded response illustrates flexibility and adaptability of the cellular signalling network, which in this case is mediated by an inducible phosphatase acting as a feedback regulator [32].

Interestingly, DUSPs also shape the dynamics of mitogenic responses. This was elegantly demonstrated by utilising a cell line which expresses a rapidly inducible Ras isoform that harbours an oncogenic, activating mutation and constitutively stimulates the downstream Raf/MEK/ERK pathway [33]. Following expression of mutated Ras, ERK activity initially overshoots but after 30 minutes rapidly reduces, resulting in a sharp activity peak. Importantly, after 30 minutes both the Ras input and ERK phosphorylation output obey a linear dose-response relationship. A mathematical model of this system showed that in order to mimic this behaviour ERK activity has to react initially in an ultrasensitive manner, but this input-output relationship subsequently changes with DUSP expression. Thus, these findings confirm the results obtained in NIH-3T3 cells, further demonstrating that expression of DUSPs affects the amplitude, dose-response relationships and temporal dynamics of MAPK activation.

Acting in this manner as rapid feedback regulator, DUSP tightly controls MAPK activity. However, computational studies suggested that if DUSP-mediated feedback is too strong, it can also bring about oscillations [22, 34]. Such oscillations have been experimentally observed in the Fus3 MAPK pathway, responsible for regulating the mating-pheromone response, in *Saccharomyces cerevisiae* [35]. Strong correlation between the oscillatory Fus3 activation peaks and periodic formation of additional mating projections suggests important physiological role of these oscillations. Experiments and mathematical modelling found that transcriptional induction of the MAPK phosphatase *Msg5* and the negative regulator of G protein signalling *Sst2* are required for maintenance of these oscillations [35].

Recent evidence further indicates that feedback control by DUSPs can shape the dynamics of the MAPK response differentially, depending on the cellular compartment [18]. In MCF7 cells the MAPK pathway responds to Heregulin (HRG) treatment with a robust and sustained activation of ERK in the cytoplasm. Surprisingly, when ERK phosphorylation is monitored in the nuclear fraction, the sustained cytoplasmic ERK signal is translated into a transient response. This holds true even if phosphorylated ERK is normalised by the amount of total nuclear ERK, taking nuclear-cytoplasmic shuttling into account. Intriguingly, knock-down of nuclear DUSPs by siRNAs is sufficient to transform HRG-induced ERK

phosphorylation within the nucleus form transient to sustained. Therefore, it appears that the difference between nuclear and cytoplasmic ERK dynamics can be due to the presence or higher expression and induction of specifically nuclear localised DUSPs.

Overall, it is becoming clear that the phosphatase-mediated deactivation of MAPK-pathways is used by the cell to control and regulate all aspects of signalling, would this be the duration, the amplitude or the localisation of the signal. Thanks to this added control the system can react with high adaptability and flexibility to changing and diverse environmental stimuli.

Spatial separation of phosphatases and kinases can give rise to phosphoprotein gradients. Cells are three-dimensional structures, and the spatial regulation of protein activities is important for many physiological processes, including cell division, motility and migration. In addition to their roles in temporal dynamics, phosphatases control the spatial behaviour of protein phosphorylation within the cell. When a protein phosphorylated at the plasma membrane spreads solely by diffusion, dephosphorylation mediated by a cytosolic phosphatase can result in a steep gradient of phosphorylation signal [4, 36]. This gradient is characterised by high concentrations of phosphorylated protein proximal to the membrane and low concentrations in the cell interior, with the decay profile being almost exponential, if the phosphatase is far from saturation [37]. Interestingly, how fast the gradient is terminated depends only on the diffusion coefficient and the apparent first-order rate constant of the phosphatase but not on the kinase. This result suggests that the localisation and catalytic activity of phosphatases can play determinant roles in shaping spatial signalling gradients in cells. Experimental evidence of such activity gradient is accumulating, which includes the small GTPase RAN [38], the yeast MAPK Fus3 [39], the phosphatase PTP1B [40], aurora B kinase [41] and the yeast protein kinase Pom1 [42]. Further work that investigated the spatial signal propagation in simplified signalling cascade models revealed similar constraints to those found for the temporal responses. For activation signals to readily spread from the cell membrane into the cell interior, the V_{max}/K_m ratios for the phosphatases must be much smaller than such ratios for the kinases [43, 44].

Drug development targeting protein phosphatases.

Kinases have been major targets of drug discovery efforts [45]. This is partly because kinases were thought to have dominant control over signalling systems, while phosphatases were considered less important counterpart of the kinases with unclear involvement in cell fate decisions, mainly as consequence of phosphatases-specific technical challenges. As discussed earlier, this view is becoming obsolete since phosphatases can impose significant influence in shaping the spatiotemporal dynamics of signalling pathways, thereby affecting cell fate decisions. Emerging systems biology approaches that combine mathematical modelling with quantitative experimentation can facilitate understanding of the network complexity and therapeutic target selection. Although the development of drugs targeting protein phosphatases is coming to the forefront, only a few of these drugs have progressed into clinical trials, and the degree of success of therapies targeting phosphatases is still to be determined. The main efforts focus on the treatment of diabetes, Alzheimer's disease and cancer. Here we summarise some of the strategies that have been used to target different classes of protein phosphatases and how systems biology can be used to develop better treatments that target phosphatases, for more detailed reviews please see [46-49].

Targeting protein tyrosine phosphatases. The protein tyrosine phosphatases are characterised by the presence of the conserved sequence (H/V)C(X)5R(s/T) in the active site [50]. Out of more than 100 PTPs, only a few are considered to be therapeutic targets [10]. For

instance, although some PTPs behave as oncogenes, an RNAi screen against 107 PTPs has shown that in HeLa cells, knock down of only four of these had a negative effect on cell survival, while knock out of 28 PTPs increased cell survival [51]. This screen shows that activation of some of these PTPs can potentially be an antitumoural treatment. However the development of drugs that specifically target a particular PTP is complicated by two factors: (i) the high level of homology of the phosphatase domains of different PTPs and (ii) the targeted sequences are highly charged, and many of the developed compounds cannot cross the membrane [47]. To increase the specificity, non-homologous neighbouring domains of the active site are also targeted. In addition, the cell permeability for drugs can be increased by chemical manipulation [47].

Drugs targeting PTPs displaying oncogenic behaviour are in different phase of development. The proteins being targeted are PTP1B, SHP2, Cdc25, Cdc14, PRL-3 and Eya1/3 [48]. Mutations of these proteins or changes in the level of expression seem to play a role in cancer and autoimmune diseases. For example, PTP1B is a negative regulator of the insulin receptor [52], and there is evidence that inhibition of this phosphatase increase sensitivity to insulin, making PTP1B a very attractive target for the treatment of obesity and diabetes [53]. Interestingly, orthovanadate was originally developed as a drug to treat diabetes, long before it was known that it inhibits PTPs. PTP1B may also positively regulate HER2 [54], subsequently activating several proteins in the downstream EGF-signalling network, such as Src [54, 55] and p120RasGAP [46]. Therefore, it may also be a potential therapeutic target for the treatment of breast cancer. PTP1B inhibitors have been developed using different approaches, the last generation are bidentate difluoromethylphosphonates designed to target the active site and a secondary substrate binding region close to the catalytic pocket [56]. These inhibitors bind PTP1B with higher affinity than other related PTPs and are being modified to increase their cell permeability [57] but have not gone into clinical trials yet. Two PTP1B inhibitors, Ertiprotanib and Trodusquemine, have advanced into clinical trials for the treatment of obesity and diabetes, although the second phase clinical trial for Ertiprotanib was discontinued due to lack of efficacy [48, 58]. Phase I clinical study of Trodusquemine is currently being performed [59]. Another PTP that has been targeted is SHP2, a phosphatase that contains two SH-2 domains [60] and is considered a *bona fide* oncogene that regulates cell progression and migration by modulating Erk1/2 and FAK signaling [7]. SHP2 is required for the full activation of ERK, and impaired SHP2 activity was found responsible for the surprising phenomenon that activating EGFR mutations failed to fully induce ERK activation [61]. Activating mutations of SHP2 have been identified in patients with different leukemias, solid tumours and in several germ line mutation syndromes such as Noonan and Leopard syndromes [62]. Gain of function mutations in the N-SH2 domain impair the auto-inhibition of the PTP domain, and usually result in increased signaling from Ras, other oncogenes such as Src, and a general increase in the downstream signal from different growth factor receptors [47]. Several SHP2 small molecule inhibitors have been produced and are in different phases of development (for a detailed review see [48]). One of the bigger problems in the development of these inhibitors is that SHP2 presents a high homology with SHP1, another PTP that acts as tumour suppressor. Thus, the SHP2 inhibitors should not inhibit SHP1, or they should have a higher affinity for SHP2 at the administration dose (Figure 1). To date no SHP2 specific inhibitors have advanced to clinical trials, however a dual SHP1/2 and PTP1B inhibitor is currently in clinical trial in combination with interferon alpha treatment. This inhibitor seems to be well tolerated and augments immunological responses [63].

Targeting protein serine/threonine phosphatases. As mentioned above, the protein Ser/Thr phosphatases (PSPs) include a variety of proteins with more than 30 catalytic subunits that interact with different regulatory and structural subunits. The PSPs actually consists of four families; the phospho-protein phosphatases (PPP), metallo-dependent protein phosphatases (PPM) and Asp-based enzymes. However of these families the PPPs are responsible for the majority of serine and threonine dephosphorylation [64]. These proteins have been shown to play an important role in the regulation of different biological functions in close relation with tyrosine kinases [65]. The PSPs are key regulators of kinase activity and their functional deregulation has been observed in different pathologies such as cancer and Alzheimer's disease. Out of many members of the PSP family, PP2A has recently become a target for drug development, specifically in the context of cancer therapy. Several isoforms of PP2A act as *bona fide* tumour suppressors which negatively regulate mitogenic signals [66], although this phosphatase is also deregulated in different types of cancer such as breast, lung and melanoma [67, 68]. Inhibition of PP2A is necessary for the transformation and tumour progression of different cancers. Both mutation and loss of expression of all PP2A subunits have been described (see [49]). In addition to the deregulation of PP2A subunits, the PP2A inhibitory proteins SET and PHAP-1 have also been linked to different malignancies. For instance, SET is overexpressed in BCR/ABL-driven leukemias [69], and PHAP-1 has been related to the aberrant phosphorylation of Tau protein in Alzheimer's disease [70]. In light of this observation different drugs that restore normal PP2A activity are being studied. For example, sodium selenate decreases the Tau protein phosphorylation levels in mice and is currently is under intense study [71]. In the context of cancer, the rationale for developing PP2A targeting drugs is that restoring the phosphatase enzymatic activity would result in inhibition of the transforming signal caused by oncogene expression. So far the best known activator of PP2A is FTY720, a structural analog of sphingosine that has been approved for the treatment of multiple sclerosis [72]. In different animal models of leukemia, FTY720 has been shown to increase the rate of survival with few toxic side effects [49, 73], indicating that PP2A activation treatment may be a safe strategy in cancer treatment. Although still in the early stages, these studies demonstrate that targeting PSPs such as PP2A is a potentially useful therapeutic strategy, however the complex spatiotemporal regulation evident within these phosphatase networks suggests that further understanding will be required to generate the sensitivity and specificity essential for therapeutic applications.

Regulatory subunits in the spatiotemporal control of phosphatases

While phosphatases, such as DUSPs, ensure the spatiotemporal regulation of pathway activity through their tight transcriptional control and internal localisation sequences, regulation of other phosphatases, such as the diverse family of PSPs, represents an entirely different paradigm of control. Distinct from the paradigm of a monomeric phosphatase, specificity and control of PSP activity (with the notable exception of the monomeric PP2Cs from the PPM family), is mediated by formation of a multi-component complex containing a catalytic subunit and a regulatory subunit. In some cases, the assembly is facilitated by a scaffolding subunit, resulting in a trimeric complex [74].

While the substrate specificity of kinases has been established upon the basis of linear motif recognition surrounding the phosphorylated amino acid, sites of PSP directed dephosphorylation do not display significant sequence similarity [75]. Instead, substrate specificity is achieved through docking of the phosphatase complex at a site distant to the dephosphorylated amino acid [12, 75]. Consensus motifs for regulatory subunit docking sites have been established for some prominent members of the PSP family, including PP1 and PP2B (Calcineurin), although not for PP2A, for which multiple interactions and post-

translational modifications play a role in directing its catalytic activity [76].

Numerous studies have demonstrated that specificity within the human PP2A network is achieved through differential assembly of heterotrimeric complexes from the genomic repertoire of two catalytic subunits (PP2A α/β), two scaffolding subunits (PR65 α/β) and at least 15 known regulatory subunits coming from four separate gene families (Termed B, B', B'' and B''') [77]. By exploiting this differential assembly mechanism PP2A exerts control over a wide array of cellular processes through the availability of a multitude of individual heterotrimeric complexes [78, 79]. Furthermore, post-translational modifications also play a significant role in the temporal regulation of the PP2A assembly which occur on both the catalytic and regulatory subunits [76]. While the incorporation of B and some B' regulatory subunits is inhibited upon Src-mediated phosphorylation of PP2A α , methylation of PP2A α may be required for the incorporation of B and possibly B' subunits [76]. Phosphorylation of regulatory subunits also contributes to this temporal regulation in a kinase and subunit specific manner [76, 80]. A further layer of spatial regulation is added to these heterotrimeric complexes through a variety of localisation sequences within the regulatory subunits, limiting the spatial sphere of PP2A activity to specific sub-cellular locales [76, 78].

A prime example of a network regulated by PP2A in such a complex manner is that governing the activation of ERK following growth factor stimulation. Within this network PP2A can act at multiple levels to promote either the activation or inhibition of ERK, depending upon the site of PP2A α recruitment, a process controlled by various regulatory subunits (Figure 2). Upon growth factor stimulation PP2A α is recruited to the KSR1/Raf1/MEK complex through the B family member PR55 α/δ , where it is required for Raf1 activation via dephosphorylation of the inhibitory S²⁵⁹ site [81] and also of 14-3-3 binding sites within Raf1 and KSR1 [82]. However, PP2A α acts via B' family member PR61 β/δ to directly inhibit ERK [83] and also indirectly promotes tyrosine dephosphorylation of Shc through an un-identified regulatory subunit [84]. Additionally, PP2A α also inhibits Ras-independent ERK activation by de-phosphorylating c-Src upon interaction with an alternative B family member PR55 γ [85].

Multi-faceted regulatory and combinatorial assembly mechanisms such as these present a significant challenge for experimental characterisation of the global PP2A network, a vital step when considering PP2A as a therapeutic target. Many studies have focused on individual complexes and their specific dephosphorylation targets, providing extensive data on how these complexes act in isolation [76-80], however little is known about regulation of PP2A at the network level.

Recent studies utilising systems level approaches have begun to yield significant advances in this field. At one level, mathematical modelling has allowed characterisation of specific PP2A heterotrimers, the abundance of which was too low to measure experimentally [86, 87], whilst interactomics based studies are beginning to piece together the PP2A network as a whole [88]. A recent study utilised mass spectrometry based interactomics to investigate the whole network of interactions occurring across PP2A catalytic, scaffolding and regulatory subunits [88]. This study confirmed the simultaneous existence of a large pool of heterogeneous heterotrimeric PP2A complexes and placed these into distinct modules characterised by the presence of regulatory subunits linked to specific cellular processes. Intriguingly, this study highlighted the underlying complexity of the PP2A network by hinting at higher order complexes containing proteins not previously associated with this network. Additionally, it also suggested the utilisation of PP2A regulatory subunits by other PSP families, demonstrating evolutionary divergence of the human PP2A network from that of lower eukaryotes.

While systems biology approaches are just starting to unravel the complex

interactions and modifications involved in regulation of the PP2A network, the building of network level protein-protein interaction networks such as this will lay the foundation for further studies examining the dynamic behaviour of these systems.

How can systems biology accelerate drug development targeting protein phosphatases?

Regulation of protein phosphorylation is seminal for many biological processes, and deregulation of kinases that catalyse phosphorylation leads to the onset of different diseases. This notion prompted the development of agents that target tyrosine kinases (TKs) years before phosphatases were considered as therapeutic targets. Most of TK targeting drugs were developed to inhibit specific kinases and were initially used as single agents, based on the idea of oncogene addiction. However apart from imatinib, most of the inhibitors that are used in the clinic have failed as single agents and they are given in combination with other treatments. This is due to an inherent biological redundancy where different TKs have overlapping targets, and inhibition of a single kinase is not sufficient to restore the normal intracellular phosphoprotein levels. Another problem is unspecific inhibition of other kinases that reduces the benefits of inhibiting a particular kinase. Hopefully, the lessons learned from the development of TK inhibitors can speed up the development of drug targeting phosphatases.

The use of mathematical models can help in the identification of appropriate targets and predict the efficiency, course of treatment and drug combinations that can have a therapeutic effect [45]. Although the use of mathematical models is still very limited in drug development there are already examples that show how systems biology can be useful for drug development and clinical application [89-91]. For example, a mathematical model of the PI3K/AKT pathway was used in combination with clinical data to identify new biomarkers that can help to decide which patients would benefit from treatment with PI3K inhibitors and receptor TK inhibitors [92]. Similar approaches have been applied to predict the drug inhibition profile for the NF κ B pathway [93], or to identify optimal therapeutic targets for the activation of p53 [94].

The complex nature of phosphatase biology makes it extremely difficult to predict off-target effects and determine which patients will benefit from treatment with particular pharmacological agents. Furthermore as mentioned, one of the challenges for the development of agents that target PTPs is their selectivity hindered by the high homology among the members of the PTP family. The use of mathematical models can help to identify “therapeutic windows” that will inhibit a given PTP without causing deleterious inhibition of other PTPs. For instance, inhibitors with a higher affinity for SHP2 than for SHP1 could be used at the doses that only affect SHP2. Furthermore, it is also likely that there will be synergistic effects upon the combination of phosphatase targeting drugs and TK inhibitors. The identification of these synergistic drug combinations may also be predicted using mathematical models.

Phosphatases-targeted therapies are suggested by a computational model of the EGFR pathway

Although efforts in targeting protein phosphatases for therapeutic purposes are already underway, this endeavour might be significantly accelerated if guided by mathematical modelling and systems approaches to cell signalling. These methods can help in the identification of suitable targets, prediction of potential drug treatments and the efficiency of combined therapies. As an illustrative example, we use a simplified mathematical model that incorporates the EGFR/ERK pathway and the tyrosine phosphatase SHP2 (the EGFR/SHP2 system) to explore alternative therapeutic strategies to inhibit ERK activation mediated by over-expressed EGFR. Our model predicts that within certain cancer

cell contexts suppression of ERK activation by targeting the phosphatase SHP2 can be more effective than targeting the receptor.

SHP2 was reported to have a dual regulatory role [61]. It negatively regulates phosphorylation of RTKs (e.g., EGFR and insulin receptor) and adaptor proteins (e.g., insulin receptor substrate (IRS) and Grb2-associated binder 1 (GAB1) [9]). Yet, SHP2 has a strong positive effect on Ras activation, facilitating the full activation of the extracellular signal-regulated kinase (ERK). This positive effect is related to the plasma membrane recruitment of SHP2 through the binding to phosphorylated tyrosine residues on the GAB1 and IRS scaffolds. SHP2 then subsequently dephosphorylates multiple docking sites, involved in the binding and membrane recruitment of the GTPase-activating protein for Ras (RasGAP), enhancing Ras activity [7, 8]. To account for the action of SHP2 on the downstream Raf-1/MEK/ERK cascade, we constructed an ODE-based model that extends our previously established EGRF network model [19] to describe the SHP2/Ras/ERK pathway. The reactions involved in the model are illustrated in the scheme presented in Figure 3 (all rate equations and parameter values are given in Supplementary Tables S1, S2 and S3). Briefly, in the EGFR/SHP2/Ras/ERK model, signal transduction is initiated by EGF binding to the extracellular domain of the monomeric EGFR (designated as R in the kinetic scheme, reaction 1, Figure 3). This causes dimerization and autophosphorylation of EGFR (reactions 2, 3 and 25), which is subsequently dephosphorylated by several phosphatases (reactions 4 and 26). To account for the combinatorial complexity of phosphorylation of different sites on EGFR and the fact that SHP2 specifically dephosphorylates the sites involved in RasGAP binding, we use an approximate ODE description by considering two separate forms of phosphorylated EGFR, designated RP_1 and RP_2 (Figure 3) (see [95-97] for more rigorous approaches to reduce combinatorial complexity of signal transduction networks). We assume that binding of proteins to these two different tyrosine residues is statistically independent. The RP_2 form mediates RasGAP-binding and is dephosphorylated by active SHP2 that has bound to RP_1 (reactions 5-8 and 27). The adaptor proteins Shc and Grb2 bind competitively to the RP_1 form, and Grb2 also binds to phosphorylated Shc (reactions 13-18). The Grb2-SOS complexes that have bound to EGFR or the EGFR-Shc complex catalyse the conversion of RasGDP to RasGTP (reaction 28), whereas the reverse transition is catalysed by RasGAP bound to the RP_2 form of EGFR (reaction 29). Activated Ras subsequently turns on the Raf-MEK-ERK cascade (reactions 30-39).

Although this dynamic model is not comprehensive, it can be exploited to compare alternative therapies that perturb distinct classes of targets. Many cancer cell types have elevated expression of EGFR [61]. Inhibiting EGFR using an EGFR inhibitor, such as gefitinib, seems to be a preferred treatment to suppress the ERK pathway activity. However, many patients show reduced gefitinib sensitivity, and new treatments that can overcome gefitinib resistance are required. Using a mathematical model, we compare two therapies that target either SHP2 (a phosphatase-based therapy) or EGFR (a kinase-based therapy).

This model shows that in normal cells, characterised by low physiological EGFR levels, EGF stimulation induces a transient response of active Ras (Ras GTP) and ERK (doubly phosphorylated ERK, designated ppERK in Fig. 3), see Figure 4A,B, which is consistent with the experimental observations. In cancer cells, EGFR overexpression often leads to sustained RasGTP and ppERK responses, which is also reflected by the model predictions (Figure 4C,D) [98]. Importantly, predictive simulations carried out using the model suggest that when the SHP2 level is also high in cancer cells, inhibition of SHP2 better suppresses active ERK and RasGTP levels compared to EGFR inhibitors (Figure 4C, D). Additionally, the model predicts that combining the two inhibitors in a dually-targeted therapy further decreases RasGTP and ppERK, thereby enhancing the efficiency of the

treatment (Figure 4C, D). For comparison purposes, the model assumed that both EGFR and SHP2 inhibitors reduce the concentrations of SHP2 and EGFR by 40% of their pre-treatment levels. Interestingly, increasing the dosage of both inhibitors not only further suppresses the Ras/EGFR pathway, but also increases the efficacy of SHP2-based therapy over the EGFR-based therapy (data not shown).

These simulations demonstrate that under certain conditions, targeting SHP2 can be a more viable strategy in suppressing ERK activation than targeting a tyrosine kinase receptor. This also highlights the emerging concept that the design of signal transduction therapies requires understanding of the underlying mechanisms that control aberrant signalling patterns and pathological cell traits. As we have demonstrated, systems biology approaches can reveal these hidden regulatory patterns and bring fresh avenues to drug discovery.

Concluding Remarks

The perception of phosphatases as enzymes whose role is solely to counteract kinases in linear signalling pipelines from receptors to target genes, is replaced by an emerging concept of a tangled kinase/phosphatase network that is tightly regulated through a multitude of negative and positive controls by feed-forward and feedback loops. Phosphorylation and dephosphorylation of multiple tyrosine, serine and threonine residues on signal transducers results in dramatic changes to their activities, leading to specific alterations in cellular phenotypes. This complex, combinatorial nature of cellular signalling highlights the need for systems biology methods to understand the roles of phosphatases in shaping the signalling dynamics and their targeting in drug development.

We are beginning to rationalise how the intricate network circuitries can determine the spatiotemporal signalling kinetics to precisely translate them into specific biological responses. We show that phosphatases can act as both immediate and delayed controllers of signal processing and how the effects of this regulation can be negative or, surprisingly, positive in amplifying cellular responses. In addition, mathematical models have shown that phosphatase and not kinase activities predominantly control the response time of distinct signalling processes and phosphorylation/dephosphorylation cascades. The prominent role of phosphatases in shaping the spatial profiles of signalling activities within a cell has been recently revealed by both computational and experimental studies.

Systems biology models emerge as a novel tool to accelerate drug development. As phosphatase targeting is coming to the forefront of drug development, there is the need to assess the systems consequences of drug-induced changes in phosphatase activities. Here we illustrate how computational modelling can help us predict the outcomes of drug therapies targeting different cellular processes. Further development of systems-level approaches will facilitate the selection of proper treatments for specific pathological conditions.

Acknowledgements

We thank Walter Kolch for stimulating discussions and reading the manuscript. This work was supported by Science Foundation Ireland under Grant No. 06/CE/B1129 and NIH grant GM059570. We apologise that we could not cite many pertinent contributions to the field because of space limitations.

References

1. Malumbres M & Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer* **9**, 153-166.
2. Waltermann C & Klipp E (2010) Signal integration in budding yeast. *Biochemical Society transactions* **38**, 1257-1264.

3. Efeyan A & Sabatini DM (2010) mTOR and cancer: many loops in one pathway. *Current opinion in cell biology* **22**, 169-176.
4. Brown GC & Kholodenko BN (1999) Spatial gradients of cellular phospho-proteins. *FEBS Lett* **457**, 452-454.
5. Heinrich R, Neel BG & Rapoport TA (2002) Mathematical models of protein kinase signal transduction. *Mol Cell* **9**, 957-970.
6. Reynolds AR, Tischer C, Verveer PJ, Rocks O & Bastiaens PI (2003) EGFR activation coupled to inhibition of tyrosine phosphatases causes lateral signal propagation. *Nat Cell Biol* **5**, 447-453.
7. Montagner A, Yart A, Dance M, Perret B, Salles JP & Raynal P (2005) A novel role for Gab1 and SHP2 in epidermal growth factor-induced Ras activation. *J Biol Chem* **280**, 5350-5360.
8. Stoker AW (2005) Protein tyrosine phosphatases and signalling. *The Journal of endocrinology* **185**, 19-33.
9. Asante-Appiah E & Kennedy BP (2003) Protein tyrosine phosphatases: the quest for negative regulators of insulin action. *American journal of physiology* **284**, E663-670.
10. Alonso A, Sasin J, Bottini N, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J & Mustelin T (2004) Protein tyrosine phosphatases in the human genome. *Cell* **117**, 699-711.
11. Van Kanegan MJ, Adams DG, Wadzinski BE & Strack S (2005) Distinct protein phosphatase 2A heterotrimers modulate growth factor signaling to extracellular signal-regulated kinases and Akt. *J Biol Chem* **280**, 36029-36036.
12. Shi Y (2009) Serine/threonine phosphatases: mechanism through structure. *Cell* **139**, 468-484.
13. Schaeffer HJ & Weber MJ (1999) Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol Cell Biol* **19**, 2435-2444.
14. Kolch W (2000) Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* **351 Pt 2**, 289-305.
15. Pages G, Lenormand P, L'Allemain G, Chambard JC, Meloche S & Pouyssegur J (1993) Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. *Proc Natl Acad Sci U S A* **90**, 8319-8323.
16. Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179-185.
17. Tombes RM, Auer KL, Mikkelsen R, Valerie K, Wymann MP, Marshall CJ, McMahon M & Dent P (1998) The mitogen-activated protein (MAP) kinase cascade can either stimulate or inhibit DNA synthesis in primary cultures of rat hepatocytes depending upon whether its activation is acute/phasic or chronic. *Biochem J* **330 (Pt 3)**, 1451-1460.
18. Nakakuki T, Birtwistle MR, Saeki Y, Yumoto N, Ide K, Nagashima T, Brusch L, Ogunnaike BA, Okada-Hatakeyama M & Kholodenko BN (2010) Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics. *Cell* **141**, 884-896.
19. Kholodenko BN, Demin OV, Moehren G & Hoek JB (1999) Quantification of short term signaling by the epidermal growth factor receptor. *J Biol Chem* **274**, 30169-30181.
20. Hornberg JJ, Bruggeman FJ, Binder B, Geest CR, de Vaate AJ, Lankelma J, Heinrich R & Westerhoff HV (2005) Principles behind the multifarious control of signal transduction. ERK phosphorylation and kinase/phosphatase control. *Febs J* **272**, 244-258.
21. Asthagiri AR & Lauffenburger DA (2001) A computational study of feedback effects on signal dynamics in a mitogen-activated protein kinase (MAPK) pathway model. *Biotechnology progress* **17**, 227-239.

22. Kholodenko BN (2000) Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur J Biochem* **267**, 1583-1588.
23. Bhalla US & Iyengar R (1999) Emergent properties of networks of biological signaling pathways. *Science* **283**, 381-387.
24. Yarden Y & Sliwkowski MX (2001) Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2**, 127-137.
25. Casci T, Vinos J & Freeman M (1999) Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**, 655-665.
26. Hackel PO, Gishizky M & Ullrich A (2001) Mig-6 is a negative regulator of the epidermal growth factor receptor signal. *Biol Chem* **382**, 1649-1662.
27. Sun H, Charles CH, Lau LF & Tonks NK (1993) MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell* **75**, 487-493.
28. Ekerot M, Stavridis MP, Delavaine L, Mitchell MP, Staples C, Owens DM, Keenan ID, Dickinson RJ, Storey KG & Keyse SM (2008) Negative-feedback regulation of FGF signalling by DUSP6/MKP-3 is driven by ERK1/2 and mediated by Ets factor binding to a conserved site within the DUSP6/MKP-3 gene promoter. *Biochem J* **412**, 287-298.
29. Owens DM & Keyse SM (2007) Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**, 3203-3213.
30. Yoon S & Seger R (2006) The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth factors (Chur, Switzerland)* **24**, 21-44.
31. Legewie S, Herzelt H, Westerhoff HV & Bluthgen N (2008) Recurrent design patterns in the feedback regulation of the mammalian signalling network. *Mol Syst Biol* **4**, 190.
32. Bhalla US, Ram PT & Iyengar R (2002) MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. *Science* **297**, 1018-1023.
33. Bluthgen N, Legewie S, Kielbasa SM, Schramme A, Tchernitsa O, Keil J, Solf A, Vingron M, Schafer R, Herzelt H & Sers C (2009) A systems biological approach suggests that transcriptional feedback regulation by dual-specificity phosphatase 6 shapes extracellular signal-related kinase activity in RAS-transformed fibroblasts. *Febs J* **276**, 1024-1035.
34. Kholodenko BN (2006) Cell-signalling dynamics in time and space. *Nat Rev Mol Cell Biol* **7**, 165-176.
35. Hilioti Z, Sabbagh W, Jr., Paliwal S, Bergmann A, Goncalves MD, Bardwell L & Levchenko A (2008) Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis. *Curr Biol* **18**, 1700-1706.
36. Lipkow K, Andrews SS & Bray D (2005) Simulated diffusion of phosphorylated CheY through the cytoplasm of Escherichia coli. *J Bacteriol* **187**, 45-53.
37. Kholodenko BN (2002) MAP kinase cascade signaling and endocytic trafficking: a marriage of convenience? *Trends in cell biology* **12**, 173-177.
38. Kalab P, Weis K & Heald R (2002) Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. *Science* **295**, 2452-2456.
39. Maeder CI, Hink MA, Kinkhabwala A, Mayr R, Bastiaens PI & Knop M (2007) Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signalling. *Nat Cell Biol* **9**, 1319-1326.
40. Yudushkin IA, Schleifenbaum A, Kinkhabwala A, Neel BG, Schultz C & Bastiaens PI (2007) Live-cell imaging of enzyme-substrate interaction reveals spatial regulation of PTP1B. *Science* **315**, 115-119.
41. Fuller BG, Lampson MA, Foley EA, Rosasco-Nitcher S, Le KV, Tobelmann P, Brautigan DL, Stukenberg PT & Kapoor TM (2008) Midzone activation of aurora B in anaphase produces an intracellular phosphorylation gradient. *Nature* **453**, 1132-1136.

42. Moseley JB, Mayeux A, Paoletti A & Nurse P (2009) A spatial gradient coordinates cell size and mitotic entry in fission yeast. *Nature* **459**, 857-860.
43. Munoz-Garcia J & Kholodenko BN (2010) Signalling over a distance: gradient patterns and phosphorylation waves within single cells. *Biochemical Society transactions* **38**, 1235-1241.
44. Munoz-Garcia J, Neufeld Z & Kholodenko BN (2009) Positional information generated by spatially distributed signaling cascades. *PLoS computational biology* **5**, e1000330.
45. Araujo RP, Liotta LA & Petricoin EF (2007) Proteins, drug targets and the mechanisms they control: the simple truth about complex networks. *Nat Rev Drug Discov* **6**, 871-880.
46. Jiang ZX & Zhang ZY (2008) Targeting PTPs with small molecule inhibitors in cancer treatment. *Cancer Metastasis Rev* **27**, 263-272.
47. Barr AJ (2010) Protein tyrosine phosphatases as drug targets: strategies and challenges of inhibitor development. *Future Med Chem* **2**, 1563-1576.
48. Scott LM, Lawrence HR, Sebti SM, Lawrence NJ & Wu J (2010) Targeting protein tyrosine phosphatases for anticancer drug discovery. *Curr Pharm Des* **16**, 1843-1862.
49. Perrotti D & Neviani P (2008) Protein phosphatase 2A (PP2A), a drugable tumor suppressor in Ph1(+) leukemias. *Cancer Metastasis Rev* **27**, 159-168.
50. Tonks NK (2006) Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* **7**, 833-846.
51. MacKeigan JP, Murphy LO & Blenis J (2005) Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nat Cell Biol* **7**, 591-600.
52. Byon JC, Kusari AB & Kusari J (1998) Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol Cell Biochem* **182**, 101-108.
53. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML & Kennedy BP (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **283**, 1544-1548.
54. Wiener JR, Kerns BJ, Harvey EL, Conaway MR, Iglehart JD, Berchuck A & Bast RC, Jr. (1994) Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression. *J Natl Cancer Inst* **86**, 372-378.
55. Zhu S, Bjorge JD & Fujita DJ (2007) PTP1B contributes to the oncogenic properties of colon cancer cells through Src activation. *Cancer Res* **67**, 10129-10137.
56. Sun JP, Fedorov AA, Lee SY, Guo XL, Shen K, Lawrence DS, Almo SC & Zhang ZY (2003) Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor. *J Biol Chem* **278**, 12406-12414.
57. Boutselis IG, Yu X, Zhang ZY & Borch RF (2007) Synthesis and cell-based activity of a potent and selective protein tyrosine phosphatase 1B inhibitor prodrug. *J Med Chem* **50**, 856-864.
58. Erbe DV, Wang S, Zhang YL, Harding K, Kung L, Tam M, Stolz L, Xing Y, Furey S, Qadri A, Klaman LD & Tobin JF (2005) Ertiprotafib improves glycemic control and lowers lipids via multiple mechanisms. *Mol Pharmacol* **67**, 69-77.
59. Roitman MF, Wescott S, Cone JJ, McLane MP & Wolfe HR (2010) MSI-1436 reduces acute food intake without affecting dopamine transporter activity. *Pharmacol Biochem Behav* **97**, 138-143.

60. Imhof D, Wavreille AS, May A, Zacharias M, Tridandapani S & Pei D (2006) Sequence specificity of SHP-1 and SHP-2 Src homology 2 domains. Critical roles of residues beyond the pY+3 position. *J Biol Chem* **281**, 20271-20282.
61. Lazzara MJ, Lane K, Chan R, Jasper PJ, Yaffe MB, Sorger PK, Jacks T, Neel BG & Lauffenburger DA (2010) Impaired SHP2-mediated extracellular signal-regulated kinase activation contributes to gefitinib sensitivity of lung cancer cells with epidermal growth factor receptor-activating mutations. *Cancer Res* **70**, 3843-3850.
62. Tartaglia M & Gelb BD (2005) Germ-line and somatic PTPN11 mutations in human disease. *Eur J Med Genet* **48**, 81-96.
63. Naing A, Reuben JM, Camacho LH, Gao H, Lee BN, Cohen EN, Verschraegen C, Stephen S, Aaron J, Hong D, Wheler J & Kurzrock R (2011) Phase I Dose Escalation Study of Sodium Stibogluconate (SSG), a Protein Tyrosine Phosphatase Inhibitor, Combined with Interferon Alpha for Patients with Solid Tumors. *J Cancer* **2**, 81-89.
64. Moorhead GB, De Wever V, Templeton G & Kerk D (2009) Evolution of protein phosphatases in plants and animals. *Biochem J* **417**, 401-409.
65. Pereira SR, Vasconcelos VM & Antunes A (2011) The phosphoprotein phosphatase family of Ser/Thr phosphatases as principal targets of naturally occurring toxins. *Crit Rev Toxicol* **41**, 83-110.
66. Sablina AA, Hector M, Colpaert N & Hahn WC (2010) Identification of PP2A complexes and pathways involved in cell transformation. *Cancer Res* **70**, 10474-10484.
67. Ruediger R, Pham HT & Walter G (2001) Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the A beta subunit gene. *Oncogene* **20**, 1892-1899.
68. Calin GA, di Iasio MG, Caprini E, Vorechovsky I, Natali PG, Sozzi G, Croce CM, Barbanti-Brodano G, Russo G & Negrini M (2000) Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene* **19**, 1191-1195.
69. Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu S, Mao H, Chang JS, Galiotta A, Uttam A, Roy DC, Valtieri M, Bruner-Klisovic R, Caligiuri MA, Bloomfield CD, Marcucci G & Perrotti D (2005) The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell* **8**, 355-368.
70. Kovacech B, Kontsekova E, Zilka N, Novak P, Skrabana R, Filipcik P, Iqbal K & Novak M (2007) A novel monoclonal antibody DC63 reveals that inhibitor 1 of protein phosphatase 2A is preferentially nuclearly localised in human brain. *FEBS Lett* **581**, 617-622.
71. Corcoran NM, Martin D, Hutter-Paier B, Windisch M, Nguyen T, Nheu L, Sundstrom LE, Costello AJ & Hovens CM (2010) Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model. *J Clin Neurosci* **17**, 1025-1033.
72. Singh M, Cugati G, Singh P & Singh AK (2011) Fingolimod: The first oral drug approved by food and drug administration; A breakthrough in treatment of multiple sclerosis. *J Pharm Bioallied Sci* **3**, 460-461.
73. Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW, Liu S, Trotta R, Muthusamy N, Gambacorti-Passerini C, Druker BJ, Cortes J, Marcucci G, Chen CS, Verrills NM, Roy DC, Caligiuri MA, Bloomfield CD, Byrd JC & Perrotti D (2007) FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest* **117**, 2408-2421.
74. Almo SC, Bonanno JB, Sauder JM, Emtage S, Dilorenzo TP, Malashkevich V, Wasserman SR, Swaminathan S, Eswaramoorthy S, Agarwal R, Kumaran D, Madegowda M,

- Ragumani S, Patskovsky Y, Alvarado J, Ramagopal UA, Faber-Barata J, Chance MR, Sali A, Fiser A, Zhang ZY, Lawrence DS & Burley SK (2007) Structural genomics of protein phosphatases. *Journal of structural and functional genomics* **8**, 121-140.
75. Roy J & Cyert MS (2009) Cracking the phosphatase code: docking interactions determine substrate specificity. *Sci Signal* **2**, re9.
76. Slupe AM, Merrill RA & Strack S (2011) Determinants for Substrate Specificity of Protein Phosphatase 2A. *Enzyme Res* **2011**, 398751.
77. Lechward K, Awotunde OS, Swiatek W & Muszynska G (2001) Protein phosphatase 2A: variety of forms and diversity of functions. *Acta Biochim Pol* **48**, 921-933.
78. Bononi A, Agnoletto C, De Marchi E, Marchi S, Patergnani S, Bonora M, Giorgi C, Missiroli S, Poletti F, Rimessi A & Pinton P (2011) Protein kinases and phosphatases in the control of cell fate. *Enzyme Res* **2011**, 329098.
79. Janssens V & Goris J (2001) Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J* **353**, 417-439.
80. Eichhorn PJ, Creighton MP & Bernards R (2009) Protein phosphatase 2A regulatory subunits and cancer. *Biochim Biophys Acta* **1795**, 1-15.
81. Abraham D, Podar K, Pacher M, Kubicek M, Welzel N, Hemmings BA, Dilworth SM, Mischak H, Kolch W & Baccarini M (2000) Raf-1-associated protein phosphatase 2A as a positive regulator of kinase activation. *J Biol Chem* **275**, 22300-22304.
82. Ory S, Zhou M, Conrads TP, Veenstra TD & Morrison DK (2003) Protein phosphatase 2A positively regulates Ras signaling by dephosphorylating KSR1 and Raf-1 on critical 14-3-3 binding sites. *Curr Biol* **13**, 1356-1364.
83. Letourneux C, Rocher G & Porteu F (2006) B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. *EMBO J* **25**, 727-738.
84. Ugi S, Imamura T, Ricketts W & Olefsky JM (2002) Protein phosphatase 2A forms a molecular complex with Shc and regulates Shc tyrosine phosphorylation and downstream mitogenic signaling. *Mol Cell Biol* **22**, 2375-2387.
85. Eichhorn PJ, Creighton MP, Wilhelmsen K, van Dam H & Bernards R (2007) A RNA interference screen identifies the protein phosphatase 2A subunit PR55gamma as a stress-sensitive inhibitor of c-SRC. *PLoS Genet* **3**, e218.
86. Witt J, Barisic S, Schumann E, Allgower F, Sawodny O, Sauter T & Kulms D (2009) Mechanism of PP2A-mediated IKK beta dephosphorylation: a systems biological approach. *BMC Syst Biol* **3**, 71.
87. Harvey SL, Enciso G, Dephoure NE, Gygi SP, Gunawardena J & Kellogg DR (2011) A phosphatase threshold sets the level of Cdk1 activity in early mitosis in budding yeast. *Mol Biol Cell*.
88. Glatter T, Wepf A, Aebersold R & Gstaiger M (2009) An integrated workflow for charting the human interaction proteome: insights into the PP2A system. *Mol Syst Biol* **5**, 237.
89. Cho CR, Labow M, Reinhardt M, van Oostrum J & Peitsch MC (2006) The application of systems biology to drug discovery. *Curr Opin Chem Biol* **10**, 294-302.
90. Belardinelli L, Shryock JC & Fraser H (2006) Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. *Heart* **92 Suppl 4**, iv6-iv14.
91. Noble D & Noble PJ (2006) Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. *Heart* **92 Suppl 4**, iv1-iv5.

92. Faratian D, Goltsov A, Lebedeva G, Sorokin A, Moodie S, Mullen P, Kay C, Um IH, Langdon S, Goryanin I & Harrison DJ (2009) Systems biology reveals new strategies for personalizing cancer medicine and confirms the role of PTEN in resistance to trastuzumab. *Cancer Res* **69**, 6713-6720.
93. Peng H, Wen J, Li H, Chang J & Zhou X (2011) Drug inhibition profile prediction for NFkappaB pathway in multiple myeloma. *PLoS One* **6**, e14750.
94. Kim DH, Rho K & Kim S (2009) A theoretical model for p53 dynamics: identifying optimal therapeutic strategy for its activation and stabilization. *Cell Cycle* **8**, 3707-3716.
95. Borisov NM, Markevich NI, Hoek JB & Kholodenko BN (2005) Signaling through receptors and scaffolds: independent interactions reduce combinatorial complexity. *Biophys J* **89**, 951-966.
96. Borisov NM, Markevich NI, Hoek JB & Kholodenko BN (2006) Trading the micro-world of combinatorial complexity for the macro-world of protein interaction domains. *Biosystems* **83**, 152-166.
97. Conzelmann H, Saez-Rodriguez J, Sauter T, Kholodenko BN & Gilles ED (2006) A domain-oriented approach to the reduction of combinatorial complexity in signal transduction networks. *BMC Bioinformatics* **7**, 34.
98. Birtwistle MR, Hatakeyama M, Yumoto N, Ogunnaike BA, Hoek JB & Kholodenko BN (2007) Ligand-dependent responses of the ErbB signaling network: experimental and modeling analyses. *Mol Syst Biol* **3**, 144.

Supplementary Material

A pdf supplementary file containing supplementary tables is available online.

Figures Legends

Figure 1. SHP-1 and SHP-2 are phosphatases that can play opposite roles in the regulation of signalling pathways. (A). In cancer, a SHP-2 activating mutation or SHP-2 stimulation by oncogenic signals results in the activation of oncogenic pathways such as the RAS/ERK and SRC pathway. (B) Inhibitors that specifically target SHP-2 or have higher affinity for SHP-2 than for SHP-1 can lead to inhibition of these pathways, shifting the balance towards tumour suppression.

Figure 2. A schematic representation of sites of PP2A activity within the network of ERK activation. Ligand-mediated activation of receptor tyrosine kinases (RTK) at the plasma membrane leads to activation of the classical Ras/Raf/MEK kinase pathway leading to phosphorylation of ERK (pathway components shown in white, activating phosphorylations indicated with a black arrow). Individual heterotrimeric PP2A complexes (shown in grey) containing the catalytic subunit (PP2aC), scaffolding subunit (PR65) and differing regulatory subunits are spatially separated within the network based upon specific interaction between network components and each regulatory subunit. The activating de-phosphorylation of network components by PP2aC is indicated by a grey arrow and inhibitory de-phosphorylation by a blunt ended grey arrow.

Figure 3. Kinetic scheme of the EGFR/SHP2 signalling mediated by adapter and target proteins. Numbering of individual steps is arbitrary.

Figure 4. A comparison of two molecularly targeted therapies for the EGFR/SHP2 signalling system as illustrated in Figure 4. Time-course concentrations of active RasGTP (**A**) and double-phosphorylated ERK (**B**) in response to EGF stimulation for normal cell with low EGFR level (100nM). Time-course concentrations of RasGTP (**C**) and double-phosphorylated ERK (**D**) in response to EGF stimulation in cancer cells, characterised by up-regulated levels of EGFR (800nM). Time-course data is also included for the presence of an EGFR inhibitor (red), SHP2 inhibitor (blue) and combined treatment of both EGFR and SHP2 inhibitors (dashed purple). Details of model equations and parameter values are given in the Supplementary Materials.

Supplementary Material

Signalling by protein phosphatases and drug development: a systems-centred view.
Lan K. Nguyen^{1*}, David Matallanas^{1*}, David R. Croucher^{1*}, Alexander von Kriegsheim^{1*} and Boris N. Kholodenko^{1#}

Table S1. Kinetic equations comprising the computational model

$R'(t) = -v_1$
$Ra'(t) = v_1 - 2v_2$
$R2'(t) = v_2 + v_4 - v_3$
$RP1'(t) = v_3 + v_7 + v_{11} + v_{15} + v_{18} + v_{20} - v_4 - v_5 - v_9 - v_{13}$
$R1SHP2'(t) = v_5 - v_6$
$R1SHP2P'(t) = v_6 - v_7$
$SHP2P'(t) = v_7 - v_8$
$SHP2'(t) = v_8 - v_5$
$R1G'(t) = v_9 - v_{10}$
$R1GS'(t) = v_{10} - v_{11}$
$R1Sh'(t) = v_{13} - v_{14}$
$R1ShP'(t) = v_{14} - v_{24} - v_{15} - v_{17}$
$R1ShG'(t) = v_{17} - v_{18} - v_{19}$
$R1ShGS'(t) = v_{19} - v_{20} + v_{24}$
$GS'(t) = v_{11} + v_{23} - v_{12} - v_{24}$
$ShP'(t) = v_{15} + v_{23} - v_{21} - v_{16}$
$ShG'(t) = v_{18} + v_{21} - v_{22}$
$ShGS'(t) = v_{20} + v_{22} - v_{23}$
$Grb'(t) = v_{12} - v_9 - v_{17} - v_{21}$
$Shc'(t) = v_{16} - v_{13}$
$SOS'(t) = v_{12} - v_{10} - v_{19} - v_{22}$
$RP2'(t) = v_{25} - v_{26} - v_{27}$
$RasGAP'(t) = -v_{27}$
$RP2RasGAP'(t) = v_{27}$
$RasGDP'(t) = -v_{28} + v_{29}$
$RasGTP'(t) = v_{28} - v_{29}$
$Raf1'(t) = -v_{30} + v_{31}$
$Raf1a'(t) = v_{30} - v_{31}$
$MEK'(t) = -v_{32} + v_{33}$
$pMEK'(t) = v_{32} - v_{33} - v_{34} + v_{35}$
$ppMEK'(t) = v_{34} - v_{35}$
$ERK'(t) = -v_{36} + v_{37}$
$pERK'(t) = v_{36} - v_{37} - v_{38} + v_{39}$
$ppERK'(t) = v_{38} - v_{39}$

Table S2. Rate equations of the kinetic model

$$\begin{aligned}v1 &= k1f * R(t) * EGF(t) - k1r * Ra(t) \\v2 &= k2f * Ra(t) * Ra(t) - k2r * R2(t) \\v3 &= k3f * R2(t) - k3r * RP1(t) \\v4 &= (V4 * RP1(t)) / (K4 + RP1(t)) \\v5 &= k5f * RP1(t) * SHP2(t) - k5r * R1SHP2(t) \\v6 &= k6f * R1SHP2(t) - k6r * R1SHP2P(t) \\v7 &= k7f * R1SHP2P(t) - k7r * RP1(t) * SHP2P(t) \\v8 &= (V8 * SHP2P(t)) / (K8 + SHP2P(t)) \\v9 &= k9f * RP1(t) * Grb(t) - k9r * R1G(t) \\v10 &= k10f * R1G(t) * SOS(t) - k10r * R1GS(t) \\v11 &= k11f * R1GS(t) - k11r * RP1(t) * GS(t) \\v12 &= k12f * GS(t) - k12r * Grb(t) * SOS(t) \\v13 &= k13f * RP1(t) * Shc(t) - k13r * R1Sh(t) \\v14 &= k14f * R1Sh(t) - k14r * R1ShP(t) \\v15 &= k15f * R1ShP(t) - k15r * ShP(t) * RP1(t) \\v16 &= (V16 * ShP(t)) / (K16 + ShP(t)) \\v17 &= k17f * R1ShP(t) * Grb(t) - k17r * R1ShG(t) \\v18 &= k18f * R1ShG(t) - k18r * RP1(t) * ShG(t) \\v19 &= k19f * R1ShG(t) * SOS(t) - k19r * R1ShGS(t) \\v20 &= k20f * R1ShGS(t) - k20r * ShGS(t) * RP1(t) \\v21 &= k21f * ShP(t) * Grb(t) - k21r * ShG(t) \\v22 &= k22f * ShG(t) * SOS(t) - k22r * ShGS(t) \\v23 &= k23f * ShGS(t) - k23r * ShP(t) * GS(t) \\v24 &= k24f * R1ShP(t) * GS(t) - k24r * R1ShGS(t) \\v25 &= k25f * R2(t) - k25r * RP2(t) \\v26 &= (V26 * RP2(t)) / (K26a + RP2(t)) + (k26a * SHP2P(t) + k26b * R1SHP2P(t)) * RP2(t) / (K26b + RP2(t)) \\v27 &= k27f * RP2(t) * RasGAP(t) - k27r * RP2RasGAP(t) \\v28 &= (k28a * R1GS(t) + k28b * R1ShGS(t)) * RasGDP(t) / (K28 + RasGDP(t)) \\v29 &= k29 * RP2RasGAP(t) * RasGTP(t) / (K29 + RasGTP(t)) \\v30 &= (k30 * RasGTP(t) * Raf1(t)) / (K30 + Raf1(t)) * (1 + Fa * (ppERK(t) / Ka)) / (1 + (ppERK(t) / Ka)) \\v31 &= (V31 * Raf1a(t)) / (Km31 + Raf1a(t)) \\v32 &= k32 * Raf1a(t) * MEK(t) / (Km32 + MEK(t) + pMEK(t) * Km32 / Km34) \\v33 &= V33 * pMEK(t) / (Km33 + pMEK(t) + ppMEK(t) * Km33 / Km35) \\v34 &= k34 * Raf1a(t) * pMEK(t) / (Km34 + pMEK(t) + MEK(t) * Km34 / Km32) \\v35 &= V35 * ppMEK(t) / (Km35 + ppMEK(t) + pMEK(t) * Km35 / Km33) \\v36 &= k36 * ppMEK(t) * ERK(t) / (Km36 + ERK(t) + pERK(t) * Km36 / Km38) \\v37 &= V37 * pERK(t) / (Km37 + pERK(t) + ppERK(t) * Km37 / Km39 + ERK(t) * Km37 / Ki) \\v38 &= k38 * ppMEK(t) * pERK(t) / (Km38 + pERK(t) + ERK(t) * Km38 / Km36) \\v39 &= V39 * ppERK(t) / (Km39 + ppERK(t) + pERK(t) * Km39 / Km37 + ERK(t) * Km39 / Ki)\end{aligned}$$

Table S3. Parameter values used in the model

Concentrations and the Michaelis-Menten constants (Kms) are given in nM. First- and second-order rate constants are expressed in s^{-1} and $nM^{-1} s^{-1}$. Maximum rates Vs are expressed in $nM s^{-1}$.

Parameters	References
k1f = 0.003, k1r = 0.06	[1]
k2f = 0.01, k2r = 0.1	[1]
k3f = 1, k3r = 0.01	[1]
V4 = 450, K4 = 50	[1]
k5f = 0.06, k5r = 0.2	[1]
k6f = 1, k6r = 0.05	[1]
k7f = 0.3, k7r = 0.006	[1]
V8 = 1, K8 = 100	[1]
k9f = 0.003, k9r = 0.05	[1]
k10f = 0.01, k10r = 0.06	[1]
k11f = 0.03, k11r = 0.0045	[1]
k12f = 0.0015, k12r = 0.0001	[1]
k13f = 0.09, k13r = 0.6	[1]
k14f = 6, k14r = 0.06	[1]
k15f = 0.3, k15r = 0.0009	[1]
V16 = 1.7, K16 = 340	[1]
k17f = 0.003, k17r = 0.1	[1]
k18f = 0.3, k18r = 0.0009	[1]
k19f = 0.01, k19r = 0.0214,	[1]
k20f = 0.12, k20r = 0.00024	[1]
k21f = 0.003, k21r = 0.1	[1]
k22f = 0.03, k22r = 0.064	[1]
k23f = 0.1, k23r = 0.021	[1]
k24f = 0.009, k24r = 0.0429	[1]
k25f = 0.1, k25r = 0	est.
V26 = 0.1, k26a = 0, k26b = 0.1, K26a = 50, K26b = 100	est.
k27f = 0.001, k27r = 1	est.
k28a = 0.1, k28b = 0.1, K28 = 100	est.
k29 = 0.1, K29 = 100	est.
k30 = 10, K30 = 100	[2]
V31 = 20, Km31 = 100	[2]
k32 = 1, Km32 = 500	[2]
V33 = 250, Km33 = 400	[2]
k34 = 5, Km34 = 200	[2]
V35 = 250, Km35 = 400	[2]
k36 = 1, Km36 = 500	[2]
V37 = 100, Km37 = 500, Ki = 1,	[2]
k38 = 10, Km38 = 500	[2]
V39 = 100, Km39 = 100	[2]
Fa = 0.1, Ka = 100	[2]
EGF(0) = 680, Grb(0) = 85, Shc(0) = 150, SOS(0) = 34	[1]
Raf1(0) = 100, MEK(0) = 200, ERK(0) = 400	[3]
GAP(0) = 200	est.
EGFR(0) = 100 for normal cell and 800 for cancer cell	est.
SHP2(0) = 300 for normal cell and 730 for cancer cell	est.

References

1. Kholodenko BN, Demin OV, Moehren G & Hoek JB (1999) Quantification of short term signaling by the epidermal growth factor receptor. *J Biol Chem* **274**, 30169-30181.
2. Kholodenko BN, Hancock JF & Kolch W (2010) Signalling ballet in space and time. *Nat Rev Mol Cell Biol* **11**, 414-426.
3. Borisov N, Aksamitiene E, Kiyatkin A, Legewie S, Berkhout J, Maiwald T, Kaimachnikov NP, Timmer J, Hoek JB & Kholodenko BN (2009) Systems-level interactions between insulin-EGF networks amplify mitogenic signaling. *Mol Syst Biol* **5**, 256.